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# Joint Quantum Chemical and Polarizable Molecular Mechanics Investigation of Formate Complexes with Penta- and Hexahydrated $\text{Zn}^{2+}$ : Comparison between Energetics of Model Bidentate, Monodentate, and Through-Water $\text{Zn}^{2+}$ Binding Modes and Evaluation of Nonadditivity Effects.

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**ABSTRACT:** In order to gain an understanding of the energetics of polycordinated  $\text{Zn}^{2+}$  binding to the formate anion (the end side chain of the Asp and Glu residues of proteins), we compare three competing binding modes in the presence of five and six water molecules: *a*, bidentate binding of  $\text{Zn}^{2+}$  to both formate oxygens; *b*, monodentate binding of  $\text{Zn}^{2+}$  to one formate oxygen; and *c*, through-water binding of  $\text{Zn}^{2+}$  to formate, in which the cation remains bound to its first-hydration shell waters and interacts with both formate oxygens through three water molecules. We also investigate a complex *d*, which is similar to *c*, in which formate is protonated into formic acid and one water molecule is deprotonated. The computations are carried out using the *ab initio*

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self-consistent field/MP2 with three basis sets of increasing size density functional theory, semiempirical AM1 and PM3, and the sum of interactions between fragments *ab initio* computed (SIBFA) molecular mechanics procedures. The summed energies of the isolated molecules making up the complexes disfavor tautomer *d* compared to *a*–*c*. On the other hand, the *ab initio* computations give the ordering of intermolecular interaction energies as *d* formic acid tautomer > *b* monodentate > *a* bidentate > *c* through-water. Whereas the first-order energy  $E_1$  favors both inner-shell  $\text{Zn}^{2+}$  complexes with formate over the outer-shell complex, the polarization and the charge-transfer components of the second-order energy  $E_2$  both favor the outer-shell complex over the inner-shell one, despite the increased separation between the cation and the highly polarizable formate ion. Energy balances including continuum solvation enthalpies produce an equilibration of complexes *a*–*d*. The preference favoring the monodentate complex over the bidentate one is consistent with other *ab initio* results for formate binding by a fully coordinated  $\text{Zn}^{2+}$  cation and with structural results from X-ray crystallography. The SIBFA results are consistent with the *ab initio* results, and the computed interaction energy values match the *ab initio* ones to within 3%. The effects of nonadditivity are analyzed in the *ab initio*, SIBFA, and semiempirical computations. © 1999 John Wiley & Sons, Inc. J Comput Chem 20: 1379–1390, 1999

**Keywords:** polycoordinated  $\text{Zn}^{2+}$ ; bidentate; monodentate; through-water; binding modes; nonadditivity effects; *ab initio*

## Introduction

Glutamate and aspartate residues occur in a recurrent fashion in the polycoordinated complexes of divalent metal cations in proteins and peptides.<sup>1</sup> In such complexes the summed contributions to the total binding energy of the second-order terms (polarization, charge-transfer at the Hartree–Fock level, and correlation at the MP2 level) can have large magnitudes. Furthermore, these contributions have a strong nonadditive character.<sup>2–5</sup> For these reasons it is necessary to resort to *ab initio* grounded molecular mechanics procedures with a separate formulation for each contribution. This led us to recently refine the sum of interactions between fragments *ab initio* computed (SIBFA) procedure.<sup>6</sup> Prior to large-scale simulations on biologically relevant complexes, we recently benchmarked this procedure by referring to high-level *ab initio* supermolecular computations on representative polycoordinated complexes of  $\text{Zn}^{2+}$  encountered in protein binding sites.<sup>5</sup> We extend these studies here by focusing on the complexes of formate (the end group of the Asp and Glu side chains) with  $\text{Zn}^{2+}$  in the presence of five and six water molecules. We compare the stabilization energies of three distinct types of complexes of inner-shell binding of  $\text{Zn}^{2+}$  to *a*, both

formate oxygens (the bidentate mode); *b*, binding to only one formate oxygen (the monodentate mode); and *c*, outer-shell coordination in which  $\text{Zn}^{2+}$  is bound to formate through three water molecules. This investigation was largely inspired by the pioneering study by Pullman et al.<sup>7</sup> that addressed a similar problem: the comparative binding energies of inner- and outer-shell complexes of tetra-, penta-, and hexahydrated  $\text{Mg}^{2+}$  with the phosphate anion. These authors showed that increasing hydration resulted in a progressive stabilization of the through-water complex, which eventually could become competitive with the monodentate mode. We also considered a complex *d* in which formate is protonated into formic acid and one water molecule is deprotonated into the hydroxy anion. The positions of  $\text{Zn}^{2+}$  and its ligating oxygens are close to those in the outer-shell complex *c*. Comparing the relative stability of *d* to *a*–*c* and how much it could be affected by the molecular environment could be important for applications to active sites of some mono- and binuclear  $\text{Zn}^{2+}$  enzymes (see, e.g., ref. 8 and refs. therein). In these, different combinations of protonation states could occur on Glu/Asp residue(s) and on water molecules of the first or second  $\text{Zn}^{2+}$  coordination shell.

We compared the values and nonadditive behaviors of the individual *ab initio* and SIBFA components of the binding energies in the different

modes. We also investigated the sensitivity of the *ab initio* results upon increasing the size of the basis set. Density functional theory (DFT) calculations using the B3LYP<sup>9</sup> functional were also carried out on these four complexes, because this procedure was shown to provide accurate results on the hydration of  $\text{Zn}^{2+}$  as compared to high-level *ab initio* computations.<sup>10</sup> This is complemented by semiempirical computations using AM1 and PM3.<sup>11</sup> The latter two procedures were evaluated in two studies. The first was on hydrogen-bonded complexes between protein side chains and/or water,<sup>12</sup> and the second was on both hydrogen-bonded and stacked complexes of the nucleic acid bases.<sup>13</sup> There is no such investigation, to our knowledge, for noncovalent complexes of  $\text{Zn}^{2+}$  with anionic ligands. Consideration of modes *a*–*d* can be essential prior to larger scale Monte Carlo simulations of the complexes of divalent cations with formate or related anions in water solution. In addition, the water ligands could also be considered as representatives for other neutral ligands completing the coordination of a formate-bound dication in model protein environments. These complexes were investigated in a recent article by Deerfield et al.,<sup>14</sup> and the essential trends from the present energy analysis should similarly apply to them.

## Procedures

We use the following basis sets: the coreless effective potential (CEP) 4-31G set derived by Stevens et al.,<sup>15</sup> supplemented on the heavy atoms by two diffuse uncontracted 3d orbitals,<sup>3</sup> which is the basis set initially used for the calibration of the SIBFA procedure; the 6-311G\*\* basis set; and the 6-311 + G(2p,2d) basis set. For  $\text{Zn}^{2+}$ , two 4f diffuse functions are included, and the overall contraction is (5111111111/51111/311/11).

The energy decompositions using the CEP 4-31G + (2d) basis set are carried out with the restricted variational space approximation method (RVS) of Stevens and Fink<sup>16</sup> using the Gamess package.<sup>17</sup> The correlation energy is computed using the MP2 procedure.<sup>18</sup> The *ab initio* computations using basis sets 2 and 3 are done using the Gaussian 94 package.<sup>19</sup>

The SIBFA intermolecular interaction energy is computed as a sum of five contributions (see ref. 6 for details):

$$\Delta E = E_{\text{MTP}} + E_{\text{rep}} + E_{\text{pol}} + E_{\text{ct}} + E_{\text{disp}},$$

which are the electrostatic, the short-range repulsion, the polarization, the charge transfer, and the dispersion contributions, respectively. The distributed multipoles<sup>20</sup> and polarizabilities<sup>21</sup> of formate and water are derived from *ab initio* calculations with the Stevens et al. basis set.<sup>15</sup> Standard geometries are used. Energy minimization on the intermolecular variables is done with the Merlin minimizer.<sup>22</sup> The bidentate mode of binding is enforced by imposing harmonic distance constraints between  $\text{Zn}^{2+}$  and the two anionic oxygens. The energy-minimized positions are used for the single-point *ab initio* self-consistent field (SCF) and MP2, as well as semiempirical computations.

The solvation energies  $\Delta H_{\text{solv}}$  are computed using the Langlet et al. continuum procedure<sup>23</sup> interfaced with SIBFA software.<sup>24</sup>

The DFT computations are done with the B3LYP functional<sup>9</sup> using the Gaussian 94 package.<sup>19</sup> The MNDO, AM1, and PM3 semiempirical computations are done using version 6 of the MOPAC code.<sup>11</sup>

## Results and Discussion

The values of the *ab initio* and SIBFA binding energies are reported in Tables I and II for the penta- and the hexahydrated complexes of  $\text{Zn}^{2+}$  with formate. These respective complexes are represented in Figures 1 and 2, which also give the relevant  $\text{Zn}^{2+}$ –formate,  $\text{Zn}^{2+}$ –water, and formate–water distances.

### PENTAHYDRATED COMPLEXES

In the inner-shell complexes *a* and *b* the coordination of  $\text{Zn}^{2+}$  is 6, whereas it is 5 in the outer-shell complex *c* and in complex *d*. Figure 1 shows that some formate–water hydrogen bonds are in the 1.6–1.7 Å range. These shortenings are an indication of the onset of local cooperative interactions occurring within the corresponding trimers formate– $\text{Zn}^{2+}$ –water.

*Ab initio* CEP 4-31G + (2d) and SIBFA computations both rank the four complexes in the following ordering of intermolecular interaction energies:

Tautomer <i>d</i> > Monodentate > Bidentate > Outer Sphere			
597.4	556.3	551.8	540.0
604.1	572.2	> 559.8	552.3
			– $\Delta E(\text{MP2})$
			– $\Delta E(\text{SIBFA})$

**TABLE I.**  
**Pentahydrated Complexes of  $\text{Zn}^{2+}$  with Formate.**

	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>
<b>Ab initio</b>				
$E_1$	-390.8	-394.6	-350.4	-432.2
$E_{\text{pol}}$	-110.4	-111.2	-131.8	-99.7
$E_{\text{ct}}$	-24.3	-25.8	-34.5	-27.5
$E_2$	-134.7	-137.0	-164.1	-126.7
$E_1 + E_2$	-525.5	-531.6	-517.7	-558.9
$\Delta E(\text{SCF})^a$	-510.9	-513.5	-500.7	-555.9
$\Delta E(\text{SCF})^b$	-505.2	-507.4	-495.0	-544.5
$E_{\text{corr}}$	-40.9	-42.8	-39.3	-41.5
$\Delta E(\text{MP2})$	-546.1	-550.2	-534.3	-586.0
<b>SIBFA</b>				
$E_1$	-410.8	-421.9	-369.4	-455.9
$E_{\text{pol}}^c$	-84.7	-81.8	-107.3	-94.9
$E_{\text{pol}}^d$	-104.7	-104.8	-124.7	-77.1
$E_{\text{ct}}$	-20.0	-20.4	-28.9	-19.9
$E_2$	-104.8	-102.2	-136.2	-97.0
$\Delta E_0$	-515.6	-524.2	-505.6	-552.9
$E_{\text{disp}}$	-44.2	-48.1	-46.6	-51.3
$\Delta E$	-559.8	-572.2	-552.3	-604.2

The table is a comparison of *a* (bidentate), *b* (monodentate), and *c* (through-water)  $\text{Zn}^{2+}$ -binding modes, and *d* (tautomer of *c*) for which formate was protonated into formic acid, and one water was deprotonated into a hydroxy.

<sup>a</sup>No BSSE correction.

<sup>b</sup>After BSSE correction.

<sup>c</sup>Including the contribution of the induced dipoles to the polarizing field.

<sup>d</sup>Without including the contribution of the induced dipoles to the polarizing field.

The SIBFA computations can match the *ab initio* ones to within < 15 kcal/mol out of 550 (i.e., to within < 3%). In both types of computations  $E_1$  is the factor imposing its preference in favor of the monodentate mode over the bidentate and the outer-shell binding modes. However, it is strongly opposed by the preferences of *both*  $E_{\text{pol}}$  and  $E_{\text{ct}}$  in favor of the outer-shell binding modes. This shows that the loss in both  $E_{\text{pol}}$  and  $E_{\text{ct}}$  that is due to the increased Zn-formate separation is compensated by a reduction of screening effects in the outer-shell arrangement and local cooperative effects. The SIBFA computations are capable of accounting for such a concerted increase of  $E_{\text{pol}}$  and  $E_{\text{ct}}$ .

Also reported are the values of  $E_{\text{pol}}^*$ (SIBFA) in which the contribution of the induced dipoles to the polarizing field, which is computed by an iterative procedure, is not included. The total non-additive behavior of the polarization energy originates from the vectorial addition of the static fields

on the one hand and from the additional effects of the induced dipoles on the other hand. In the present case, these will reinforce the negative fields at the ligand sites. We observe  $E_{\text{pol}}^*$ (SIBFA) to be in good numerical agreement with  $E_{\text{pol}}$ (SCF) calculated using the RVS analysis. This translates to a consistency between the representation of the polarization energy by this analysis, where each ligand is polarized by the field due to the unperturbed electronic distribution of the other ligands, and that of  $E_{\text{pol}}^*$ (SIBFA) in which such a field is computed with their sole permanent distributed *ab initio* multipoles.

In contrast to the situation encountered in the water aggregates,<sup>6f</sup>  $E_{\text{corr}}$ (MP2) has a pronounced nonadditive behavior as analyzed below. At the MP2 level there are changes in the electronic properties of the monomers, which will affect the individual components of the *ab initio* binding energy with respect to the SCF uncorrelated case.<sup>25</sup> These effects are to be added to the actual "dispersion" term. They are not captured by SIBFA. In the present formulation, electronic redistribution effects due to correlation are not included in the expression of  $E_{\text{disp}}$ ; the other components of  $\Delta E$  are computed with multipoles and polarizabilities derived from SCF wave functions for consistency in order to make a comparison with the RVS calculations. Future refinements will integrate multipoles derived from correlated density matrices. Thus, at present a numerical evaluation of  $E_{\text{disp}}$ (SIBFA) by comparison with  $E_{\text{corr}}$ (MP2) has to be done with caution. Nevertheless, it can be observed that adding  $E_{\text{disp}}$ (SIBFA) to  $\Delta E_0$ (SIBFA) to provide  $\Delta E$ (SIBFA) does not seriously impair the match to  $\Delta E$ (MP2) as compared to the uncorrelated level situation. For the pentahydrates, the reason for the overestimated values of  $E_{\text{disp}}$ (SIBFA) with respect to  $E_{\text{corr}}$ (MP2) is largely due to the nonadditivity of  $E_{\text{corr}}$ .

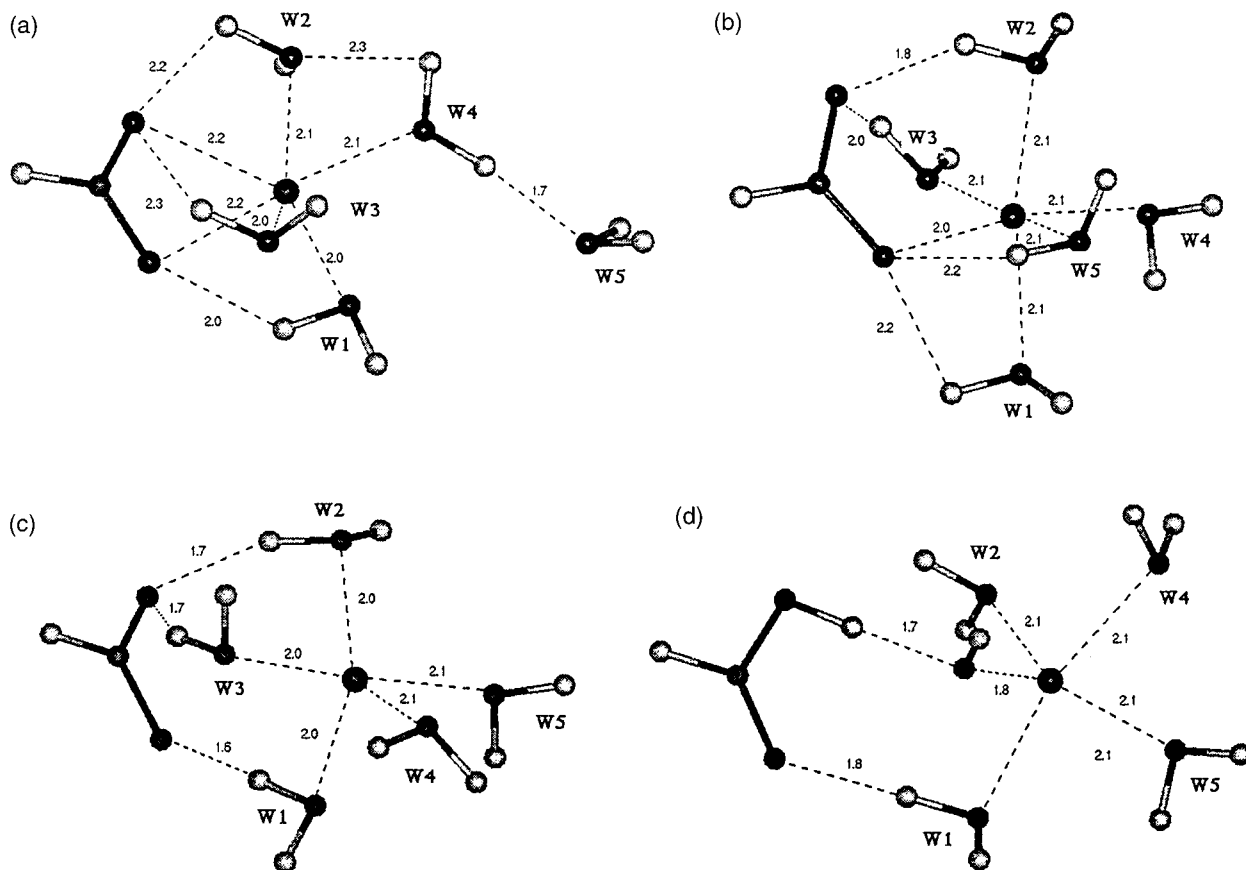
The energy difference between *ab initio* mono- and bidentate structures (4 kcal/mol out of 550) is small. *Ab initio* energy minimization of these complexes could not be carried out, so the preference favoring the monodentate over the bidentate structure cannot be considered as definite. The magnitudes of the binding energies and the binding energy differences may also be sensitive to the size and flexibility of the basis sets used in the *ab initio* computations. In this connection, recent studies by Rotzinger,<sup>26</sup> Hartmann et al.,<sup>10a</sup> and Pavlov et al.<sup>10b</sup> using extended basis sets showed the preferred coordination number of  $\text{Zn}^{2+}$  for a shell of water

**TABLE II.**  
**Comparison between Stabilities of Complexes *a* – *d* with Basis Sets of Increasing Size.**

	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>
Uncorrelated calculations				
Binding energies				
CEP 4-31G + (2d)	–510.8	–513.5	–500.6	–555.9
6-311G**	–529.3	–533.5	–521.4	–580.4
6-311 + G(2d,2p)	–503.2	–505.1	–493.4	–540.3
SIBFA calculations	–515.6	–524.2	–505.6	–552.9
Differences between SCF energies of isolated monomers				
CEP 4-31G + (2d)	0.0	0.0	0.0	57.1
6-311G**	0.0	0.0	0.0	61.6
6-311 + G(2d,2p)	0.0	0.0	0.0	48.2
Differences between SCF global energies of complexes				
CEP 4-31G + (2d)	2.7	0.0	12.9	14.8
6-311G**	4.1	0.0	12.1	14.7
6-311 + G(2d,2p)	1.8	0.0	11.7	14.9
SIBFA	8.6	0.0	18.6	28.4
Correlated calculations				
Binding energies				
CEP 4-31G + (2d)	–551.7	–556.3	–540.0	–597.4
6-311G**	–568.2	–572.3	–559.2	–622.5
6-311 + G(2d,2p) / B3LYP	–535.8	–535.8	–528.4	–573.4
SIBFA calculations	–559.8	–572.2	–552.2	–604.1
Differences between MP2 or DFT energies of isolated monomers				
CEP 4-31G + (2d)	0.0	0.0	0.0	59.1
6-311G**	0.0	0.0	0.0	65.3
6-311 + G(2d,2p)	0.0	0.0	0.0	46.1
Differences between MP2 global energies of complexes				
CEP 4-31G + (2d)	4.6	0.0	16.3	18.0
6-311G**	4.1	0.0	13.4	15.6
6-311 + G(2d,2p) / B3LYP	0.0	0.0	7.4	8.5
SIBFA	12.4	0.0	20.0	27.2

molecules to be basis-set sensitive. Tautomer *d* differs from complexes *a*–*c* by the replacement of formate and one water by formic acid and a hydroxy. A comparison between the *total* stabilities of *a*–*c* versus that of *d* needs to include in the energy balances the summed energies of the individual isolated monomers making up the complex. Such energies can be very sensitive to the size of the basis set. These considerations led us to reinvestigate complexes *a*–*d* by resorting to two extended, full-electron basis sets, namely, 6-311G\*\* and 6-311G + (2d,2p). Because of the size of the latter set, the calculations could not be carried out with it at the MP2 level. They were performed instead by DFT computations using the B3LYP functional. This was motivated by recent studies on  $\text{Zn}^{2+}$  hydration that showed that DFT/B3LYP

with high-quality basis sets provided accurate binding energies with respect to *ab initio* computations.<sup>10</sup> The results of the comparisons are reported in Table II. The basis-set superposition error (BSSE) correction was not taken into account at this stage. At uncorrelated and correlated levels, Table II reports the intermolecular interaction energies, the differences ( $\delta_0$ ) in the summed energies of the isolated monomers making up the complex, and the differences ( $\delta_T$ ) in total energies of the complexes with respect to complex *b*. Molecular mechanics is not designed to predict proton transfer energetics that involve the breakage and formation of covalent bonds. In order to evaluate the extent of this limitation, we also included the result of an attempt to perform a similar energy balance with SIBFA. Thus, Table II also reports the



**FIGURE 1.** Representation of the pentahydrated complexes of  $\text{Zn}^{2+}$  with formate: (a) bidentate, (b) monodentate, (c) through-water, (d) tautomer of c; formate was protonated into formic acid, and one water was deprotonated into a hydroxy.

values of the interaction energies using this method, and we subtract the values of  $\delta_0$  using the CEP 4-31G + (2d) basis set.

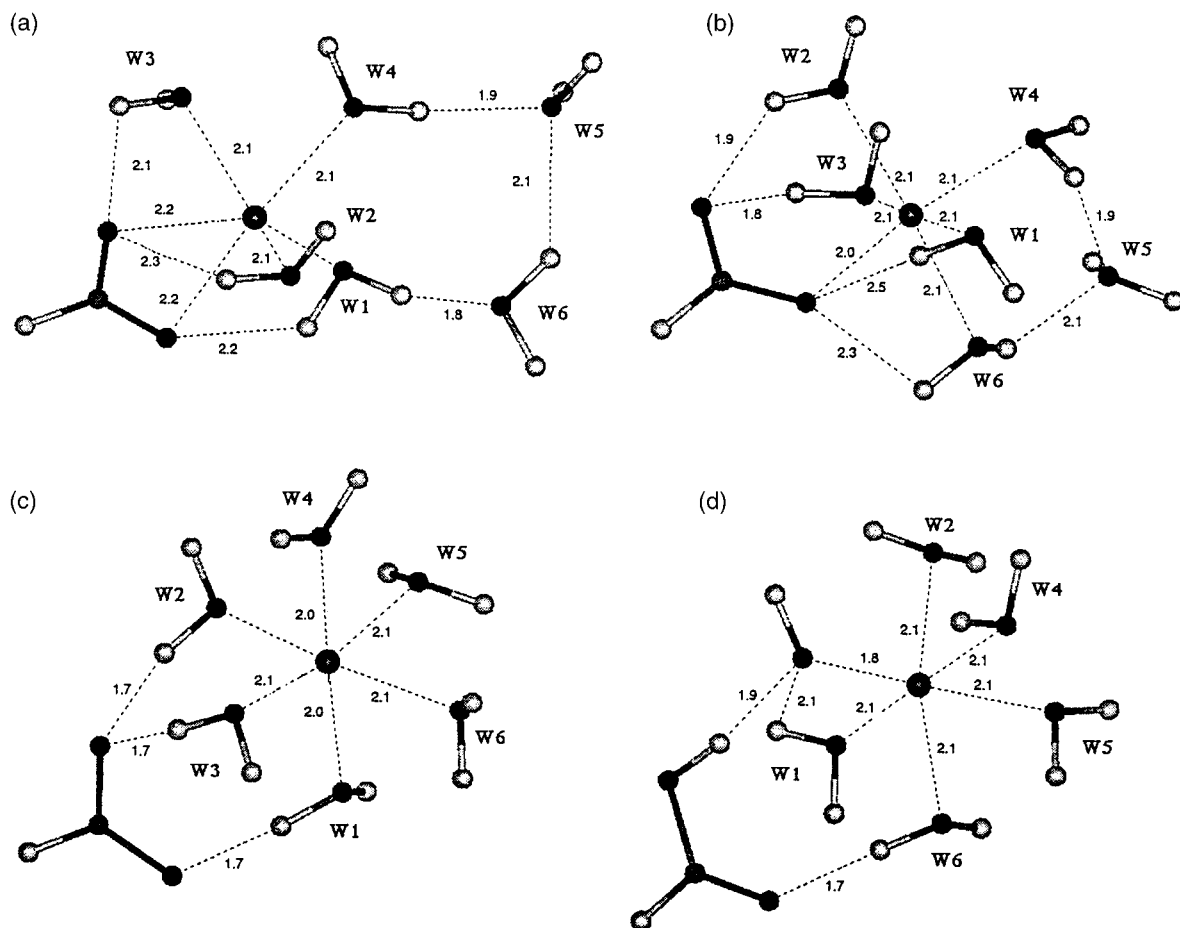
### Uncorrelated Calculations

All three basis sets produce the same  $\Delta E$  ordering along the  $d > b > a > c$  sequence. The values with the 6-311G\*\* basis set are about 20 kcal/mol out of 530 larger than with the CEP 4-31G + (2d) set for complexes *a*–*c* and 35 kcal/mol larger for *d*. The values with the more extended 6-311 + G(2d,2p) set are much closer to the CEP 4-31G + (2d) set than to the 6-311G\*\* one. The energy difference now amounts to 8 kcal/mol in *a*–*c* and 15 kcal/mol in *d*, which is thus reduced to 1.6–3% of the interaction energies. The summed energies of the isolated monomers disfavor tautomer *d* with respect to *a*–*c* by  $\delta_0$  values in the 48–62 kcal/mol range according to the basis set. This is opposed by the fact that tautomer *d* has the strongest  $\Delta E$

values. In terms of overall energy balances, all three SCF computations consistently give tautomer *d* as being disfavored with respect to *b* by a  $\delta_T$  of 15 kcal/mol on the one hand and by only 2–3 kcal/mol with respect to the through-water complex *c* on the other hand. In the SIBFA computations using the same value of  $\delta_0$  as in the CEP 4-31G + (2d) basis set, the ordering  $b > a > c > d$  is retained, but the values of  $\delta_T$  are larger than in the *ab initio* computations.

### Correlated Calculations

The 6-311G\*\* basis set ranks all four complexes in the same order as the CEP 4-31G + (2d) one. It provides  $\Delta E$  values that are larger than the CEP 4-31G + (2d) ones by amounts similar to those computed at the SCF level. The comparable values of  $E_{\text{corr}}$  found with both basis sets show that at the MP2 level BSSE effects are not magnified with the CEP 4-31G + (2d) set as compared to the 6-311G\*\*



**FIGURE 2.** Representation of the hexahydrated complexes of  $\text{Zn}^{2+}$  with formate: (a) bidentate, (b) monodentate, (c) through-water, (d) tautomer of c; formate was protonated into formic acid, and one water was deprotonated into a hydroxy.

one. Both sets give comparable  $\delta_0$  and  $\delta_T$  values. The DFT/B3LYP computations with the 6-311 + G(2d,2p) basis set yield values of  $\Delta E$  that are smaller than the CEP 4-31G + (2d) ones by 12–24 kcal/mol. This offset is larger than the one found without correlation. We note that the mono- and bidentate complexes are equalized in energy in these single-point DFT computations. These DFT computations also give *c* and *d* as having twofold reduced values of  $\delta_T$  with respect to the MP2 computations whereas those from the SIBFA computations are overestimated.

### HEXAHYDRATED COMPLEXES

We wished to undertake a preliminary evaluation of the effects of the environment (solvation) on the relative stabilities of complexes *a–d*. Solvation effects modeled by a continuum reaction field

procedure are more meaningful if the first-shell coordination of  $\text{Zn}^{2+}$  for all complexes is completed by at least six ligands. We report in Table III the results of SIBFA energy minimizations on the complexes of  $[\text{Zn}(\text{H}_2\text{O})_6]^{2+}$  with formate illustrated in Figure 2. In *a* and *b* two and one water molecules, respectively, are in the second-shell coordination of  $\text{Zn}^{2+}$ . As was the case in the  $[\text{Zn}(\text{H}_2\text{O})_5]^{2+}$  complexes, Figure 2 shows cooperativity-induced shortenings of the formate–water hydrogen bonds for *c* and *d*.

The  $\Delta E(\text{SCF})$  and  $\Delta E_0(\text{SIBFA})$  retain the ranking  $d > b > a > c$ . The MP2 calculations have the same ordering as the SCF ones whereas  $E_{\text{disp}}$  in the SIBFA calculations produces a small (2.3 kcal/mol) inversion in favor of the outer-shell complex relative to the bidentate one. Nevertheless, the total values of the SIBFA binding energies remain able to numerically match the *ab initio* ones

**TABLE III.**  
**Hexahydrated Complexes of Zn<sup>2+</sup> with Formate.**

	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>
$\delta_{0(\text{MP2})}$	0.0	0.0	0.0	59.1
<i>Ab initio</i>				
$\Delta E(\text{SCF})^a$	-524.3	-530.3	-517.4	-570.4
$E_{\text{corr}}$	-45.6	-46.9	-46.1	-47.2
$\Delta E(\text{MP2})$	-569.9	-577.2	-563.5	-617.6
$\delta_{\text{T}(\text{MP2})}$	7.3	0.0	13.7	18.7
<b>SIBFA</b>				
$\Delta E_0$	-527.8	-539.6	-524.9	-571.7
$E_{\text{disp}}$	-47.0	-51.2	-52.2	-55.7
$\Delta E$	-574.8	-590.9	-577.1	-627.4
$\delta T$	16.1	0.0	13.8	22.6
<b>Energy balances<sup>b</sup></b>				
$\Delta H_{\text{solv}}$	-98.8	-93.8	-105.0	-107.7
$\Delta E_{\text{MP2}} + \Delta H_{\text{solv}}$	-668.7	-671.0	-668.5	-725.1
$\delta_{\text{MP2}} + \delta_{\text{solv}}$	2.3	0.0	2.5	-54.3
$\Delta E_{\text{SIBFA}} + \Delta H_{\text{solv}}$	-673.6	-684.7	-682.1	-735.1
$\delta_{\text{SIBFA}} + \delta_{\text{solv}}$	11.1	0.0	2.6	-50.4
<b>Energy balances<sup>c</sup></b>				
$\delta_0$	0.0	0.0	0.0	59.1
$\delta_{\text{MP2}} + \delta_{\text{solv}} + \delta_0$	2.3	0.0	2.5	4.8
$\delta_{\text{SIBFA}} + \delta_{\text{solv}} + \delta_0$	11.1	0.0	2.6	8.7

The table is a comparison of *a* (bidentate), *b* (monodentate), and *c* (through-water) Zn<sup>2+</sup>-binding modes, and *d* (tautomer of *c*); formate was protonated into formic acid, and one water was deprotonated into a hydroxy.

<sup>a</sup>Uncorrected for BSSE.

<sup>b</sup>With the complexation and solvation enthalpies.

<sup>c</sup>Including the summed energies of the isolated monomers, the intermolecular interaction energies, and the solvation enthalpies.

to within 14 kcal/mol out of 570, which is an error of < 3%. The bidentate complex undergoes a further destabilization with respect to the monodentate one. Thus,  $\delta_{\text{T}(\text{MP2})}$  amounts to 7.3 kcal/mol as compared to 4.6 in the pentahydrates. Conversely, the outer-shell complex has a reduced  $\delta_{\text{T}}$  of 13.8 kcal/mol versus 16.3 in the pentahydrates.

The preferential stabilization of the mono- over the bidentate mode of binding to formate upon saturation of the coordination shell is consistent with the survey by Carrell et al.<sup>1</sup> showing the prevalence of the binding of divalent cations to carboxylate along the syn position in crystal structure determinations. A study by Krauss et al. comparing the direct and through-water binding of platinum tetraammine to the phosphate anion showed both modes to be competitive,<sup>27</sup> whereas in the present computations and in the absence of continuum solvation direct binding of Zn<sup>2+</sup> to

formate remains favored over the through-water binding mode. The equilibration occurring in the Pt ammine-phosphate complexes could reflect the destabilization of the direct binding mode due to the delocalization of the dipositive charge of Pt on its four ammine ligands, as well as a partial hindrance of the direct Pt-O contact due to them.

Single-point computation of the solvation enthalpies of *a-d* are done using the Langlet-Claverie reaction field procedure.<sup>23</sup> This procedure uses the same distributed *ab initio* multipoles as in SIBFA, so that both should be mutually consistent. Although a more thorough evaluation would clearly request embedding *a-d* in an actual water bath and Monte Carlo configuration sampling, the present results already indicate the following trends.  $\Delta H_{\text{solv}}$  is larger for the outer-shell complex *c* than either of the inner-shell ones *a* or *b*, which results in improved stabilization of *c*. Thus, with both *ab initio* and SIBFA computations *c* is less stable than the best-bound inner-shell complex *b* by only 2.6 kcal/mol out of 680. Inclusion of the summed energies of the individual monomers shows tautomer *d* to be the least favored complex. By contrast,  $\Delta H_{\text{solv}}$  being the largest for *d*, its inclusion in the MP2 energy balances produces an equilibration of all four complexes *a-d*, now seen to differ from one another by less than 4.8 kcal/mol out of 730. Such energy balances are to be considered as preliminary, because a relaxation of the energy surface is warranted, which would include the tunneling of the proton between the formate-formic acid and the water-hydroxy ligands. Nevertheless, the present results imply that environmental effects should be fully capable of equilibrating inner-shell, outer-shell, and the formic acid-hydroxy tautomer complexes. The global energy balances using SIBFA are consistent with the *ab initio* ones, although the  $\delta_{\text{T}}$  values remain larger. Nevertheless, they differ from the *ab initio* ones by smaller overall amounts compared to the pentahydrated level.

## ANALYSIS OF NONADDITIVITY EFFECTS

Nonadditivity is a crucial feature of polycordinated complexes of divalent cations<sup>2-5,28</sup> and multiply hydrogen-bonded complexes (refs. 6f, 29, and 30 and refs. therein). The analysis was carried out for complexes *a* and *b*. It could not be carried out for the outer-shell complex *c*, because the corresponding *binary* complex between formate and Zn<sup>2+</sup> converged toward the different electronic



configuration of an open-shell one with one electron from formate transferred to  $\text{Zn}^{2+}$ . The occurrence of such states was discussed in our previous work<sup>6d</sup> and noted in an early article by Corongiu and Clementi.<sup>31</sup> Although they prevent the analysis of nonadditivity effects in  $c$ , these states are considered as not relevant for actual simulations, because the closed-shell configurations are the ones stabilized by the SCF procedure when there are ligand(s) interposed between formate and  $\text{Zn}^{2+}$ . Table IV compares the values of  $E_1$ ,  $E_{\text{pol}}$ ,  $E_{\text{ct}}$ , and  $E_{\text{corr}}/E_{\text{disp}}$  summed in the binary complexes as compared to those in the formate- $\text{Zn}^{2+}$  pentahydrated complexes. The values of these components in each of the individual binary complexes are given as Supplementary Material Table IV\*. The most salient features follow.

With *ab initio* and SIBFA computations the summed nonadditivity (denoted as  $\delta E_{\text{nadd}}$  below) is larger in the monodentate complex than in the bidentate one.  $E_{\text{pol}}$  gives rise to the largest  $\delta E_{\text{nadd}}$  values. The charge transfer energy gives rise to the second largest values of  $\delta E_{\text{nadd}}$  in the range of 39–50 kcal/mol. The nonadditivity of  $E_{\text{ct}}$  results in this component having closely similar values in the complexes of formate with this cation, whether bare or in the presence of five water molecules.

In the absence of nonadditivity effects, the summed values of  $\Delta E_0(\text{SIBFA})$  reproduce the corresponding values of  $\Delta E(\text{SCF})$  to within 4 kcal/mol out of 700 in both complexes. This is due to the close agreement at the level of each of the individual binary complexes illustrated in Table IV\*. However, this agreement does not hold when comparing  $E_{\text{disp}}(\text{SIBFA})$  to  $E_{\text{corr}}(\text{MP2})$ ; the SIBFA values are systematically smaller than the

MP2 ones, particularly for the complexes of bare  $\text{Zn}^{2+}$  with its individual ligands. For the latter complexes this is due to a faster radial decay of  $E_{\text{disp}}$  than  $E_{\text{corr}}$  for Zn–O distances larger than the equilibrium distances at which  $E_{\text{disp}}$  was initially calibrated<sup>6d</sup> and that are 0.2 Å shorter than those in the actual pentahydrates. Nevertheless, in the pentahydrate complexes this is compensated by the values of  $\delta E_{\text{nadd}}(E_{\text{corr}})$  amounting to 37.6 and 44.8 kcal/mol in the bi- and monodentate complexes. These result in  $E_{\text{disp}}$  having slightly greater values than  $E_{\text{corr}}$ .

$E_1(\text{SCF})$  has a nonadditive character, although it does not exceed 8–10 kcal/mol out of 400. Such a nonadditive character of  $E_1(\text{SCF})$  cannot be accounted for in molecular mechanics. Much smaller values were computed in the complexes of  $\text{Zn}^{2+}$  with two to four anionic ligands.<sup>28</sup>

## COMPARISON WITH SEMIEMPIRICAL QUANTUM CHEMICAL COMPUTATIONS

Large molecular complexes of 50 and more atoms are not easily amenable to *ab initio* computations using extended basis sets. Thus, for such complexes an alternative to polarizable molecular mechanics consists of semiempirical quantum chemical procedures such as MNDO, AM1, or PM3. They were used in the earliest “hybrid” QM/MM procedures interfacing a quantum chemical core with a molecular mechanics surrounding<sup>32</sup> and to investigate the tautomeric states in a model for the  $\text{Zn}^{2+}$  binding site in thermolysin.<sup>33</sup> Similar to the *ab initio* computations, only single-point computations are done at the SIBFA optimized positions in order to evaluate the nonadditive behavior of the

**TABLE IV.**  
Determination of Nonadditive Behavior of Components of *Ab Initio* and SIBFA Binding Energies for Bi- and Monodentate Complexes of  $\text{Zn}^{2+}$  Pentahydrate (kcal / mol).

	$E_1$		$E_{\text{pol}}$		$E_{\text{ct}}$		$\Delta E_0$	$\Delta E(\text{SCF})$	$E_1 + E_2$	$E_{\text{disp}}$	$E_{\text{corr}}$	
	SIBFA	SCF	SIBFA	SCF	SIBFA	SCF	SIBFA	SCF	SCF	SIBFA	MP2	
Bidentate			(a)	(b)								
Sigma	−411.0	−399.2	−192.7	−192.7	−207.9	−70.4	−63.4	−674.2	−675.4	−670.2	−44.3	−78.5
$\Delta E$	−410.8	−390.8	−84.7	−104.7	−110.4	−20.0	−24.3	−515.6	−505.2	−525.5	−44.2	−40.9
$\delta E_{\text{nadd}}$	0.0	8.4	108.0	88.0	97.5	50.4	39.1	158.6	170.2	144.7	0.0	37.6
Monodentate												
Sigma	−421.9	−405.2	−213.7	−213.0	−232.1	−78.4	−71.5	−714.0	−718.3	−708.4	−47.8	−87.6
$\Delta E$	−421.9	−394.6	−81.8	−104.8	−111.2	−20.4	−25.8	−524.2	−507.4	−531.6	−48.0	−42.8
$\delta E_{\text{nadd}}$	0.0	10.6	131.9	108.2	120.9	50.0	45.7	189.8	210.9	176.8	0.0	44.8

(a)  $E_{\text{pol}}$   
(b)  $E_{\text{pol}}^*$

**TABLE V.**  
**Penta- and Hexahydrated Formate – Zn<sup>2+</sup> Complexes.**

	Bidentate	Monodentate	Outer Shell	Formic Acid Tautomer
Pentahydrated complexes				
$\Delta E(\text{SCF}, \text{AM1})$	–485.0	–482.6	–457.2	–529.4
$\delta / \text{monodentate}$	–2.4	0.0	25.4	–46.8
$\Delta E(\text{SCF}, \text{PM3})$	–419.6	–418.0	–390.2	–476.9
$\delta / \text{monodentate}$	–1.9	0.0	17.8	–58.9
$\Delta E(\text{SCF}, \text{ab initio})$	–505.2	–507.4	–495.0	–544.5
$d / \text{monodentate}$	1.8	0.0	12.4	–37.1
$\Delta E0(\text{SIBFA})$	–515.6	–524.2	–505.6	–552.9
$\delta / \text{monodentate}$	8.6	0.0	18.6	–28.7
Hexahydrated complexes				
$\Delta E(\text{SCF}, \text{AM1})$	–494.6	–492.0	–461.7	–540.5
$\delta / \text{monodentate}$	–2.6	0.0	30.3	–48.5
$\Delta E(\text{SCF}, \text{PM3})$	–424.1	–426.8	–407.8	–482.0
$\delta / \text{monodentate}$	2.7	0.0	19.0	–55.2
$\Delta E(\text{SCF}, \text{ab initio})$	–524.3	–530.3	–517.4	–570.4
$d / \text{monodentate}$	6.0	0.0	12.9	–40.1
$\Delta E0(\text{SIBFA})$	–527.8	–539.6	–524.9	–571.7
$\delta / \text{monodentate}$	11.8	0.0	14.7	–32.1

The table is a comparison of the results of *ab initio* SCF, semiempirical quantum chemical, and SIBFA molecular mechanics computations.

semiempirical procedures, because these are not expected to vary significantly in the neighborhood of these energy minima. Because correlation effects are not implemented, the comparisons are done with  $\Delta E(\text{SCF})$ . Table V reports the values of the AM1 and PM3 binding energies for the penta- and hexahydrated complexes, and Table VI reports the nonadditivities. The decomposition into a sum of binary interactions is given as Supplementary Material Table VI\*. Both procedures show that the mono- and bidentate modes differ by < 1% of their  $\Delta E$  values. They underestimate the relative stability of the outer-shell complex and overestimate that of tautomer *d*. AM1 and PM3 reproduce  $\Delta E(\text{SCF})$  with relative errors of 12 and 20%, respectively. AM1 gives rise to very large nonaddi-

tivities, the values of  $\delta E_{\text{nadd}}$  being more than twice as large as the corresponding *ab initio* values. The PM3 computations yield values of  $\delta E_{\text{nadd}}$  that are much closer to the *ab initio* ones (198.4 and 238.7 kcal/mol in the bi- and monodentate complexes, respectively) compared to the corresponding *ab initio* values of 170.2 and 210.9. This implies that the underestimated values of  $\Delta E(\text{PM3})$  in complexes *a*, *b*, and *c* with respect to  $\Delta E(\text{SCF}, \text{ab initio})$  stems from the underestimation of the monoligated complexation energies of Zn to its ligands, in particular the  $\text{Zn}^{2+}\text{—H}_2\text{O}$  complexes. It is suggested that recalibrating the Zn parameters in PM3 to reproduce *ab initio* results on model binary complexes could significantly improve the agreement with *ab initio* results on polycordinated complexes of  $\text{Zn}^{2+}$ .

**TABLE VI.**  
**Pentahydrated Formate – Zn<sup>2+</sup> Complexes.**

	Bidentate			Monodentate		
	AM1	PM3	Ab Initio	AM1	PM3	Ab Initio
Sigma	–886.0	–618.0	–675.4	–939.9	–656.7	–718.3
$\Delta E$	–485.0	–419.6	–505.2	–482.6	–418.0	–507.4
$\delta E_{\text{nadd}}$	401.0	198.4	170.2	457.3	238.7	210.9

The table is a comparison of the AM1, PM3 and *ab initio* results. Energies of the binary complexes and evaluation of nonadditivity effects.

## Conclusions

In this study we compared the binding energies of formate as a model for the side chains of Asp and Glu protein residues to hydrated  $\text{Zn}^{2+}$  in three competing binding modes: bidentate and monodentate binding to the anionic oxygens and outer shell. For the latter we also investigated the tautomeric structure having formate protonated

into formic acid and one water molecule deprotonated into a hydroxy. For both  $\text{Zn}^{2+}$  penta- and hexahydrates, the *ab initio* and SIBFA computations indicate the monodentate binding mode as preferred over the bidentate one. This preference is consistent with the conclusion from a compilation of crystallographic data showing that the binding of  $\text{Zn}^{2+}$  to the formate moiety is favored in the monodentate mode over the bidentate mode.<sup>1</sup> The outer-shell binding mode has less stability than that of the bidentate mode, but the energy difference decreases about twofold in the *ab initio* computations upon passing from the penta- to the hexahydrate. Inclusion of solvation effects<sup>23</sup> results in energy balances differing by only 2.6 kcal/mol between the inner-shell monodentate complex and the outer-shell one.

An essential objective of this work was to evaluate the relative weights of the first- and second-order components of  $\Delta E$  in the competing complexes and to quantify the nonadditivity effects. At the SCF level,  $E_1$  is the sole  $\Delta E$  component responsible for the preference in favor of the monodentate complex over the through-water one.  $E_{\text{pol}}$  and  $E_{\text{ct}}$  both have a strong preference in favor of the outer-shell complex. This indicates that reduction of screening effects of the polarizing field, and local cooperative Zn–water–formate effects in the outer-shell complex can compensate for the increased  $\text{Zn}^{2+}$ –formate separation occurring with respect to the inner-shell complexes. The SIBFA computations are capable of reproducing the behaviors of the components of the *ab initio* binding energies and matching the values of  $\Delta E(\text{SCF})$  and  $\Delta E(\text{MP2})$  to within 3%. Nonadditivity was analyzed in the bi- and monodentate Zn–pentahydrate complexes.  $E_{\text{pol}}$  and  $E_{\text{ct}}$  have strong nonadditive characters, and their values are reduced about two- and threefold, respectively, compared to their summed pairwise values. In addition, and contrary to the case of multiply hydrogen-bonded complexes of neutral molecules (refs. 6f and 30 and refs. therein),  $E_{\text{corr}}(\text{MP2})$  is also significantly nonadditive. This indicates that further refinements in molecular mechanics should be able to confer a nonadditive character to  $E_{\text{disp}} \cdot \delta E_{\text{nadd}}(E_1)$  was limited to < 10 kcal/mol out of 400.

These joint *ab initio* and SIBFA computations could also be used to benchmark new polarizable molecular mechanics potentials. For that purpose we provide as Supplementary Material the coordinates of the four pentahydrated complexes.

Complexes *a*–*c* were also investigated using the semiempirical quantum chemical procedures AM1 and PM3.<sup>11</sup> Of these two procedures, the best numerical agreement with the *ab initio* SCF results (12%) is afforded by the AM1 procedure. However, the hexahydrated outer-shell complex is substantially more disfavored with respect to the monodentate one than was the case with the *ab initio* computations (30.3 kcal/mol instead of 12.9). A considerably magnified amplitude of nonadditivity in complexes *a* and *b* is found in these AM1 computations compared to *ab initio*. The PM3 computations display, on the other hand, amplitudes of nonadditivity that are virtually identical to the *ab initio* ones in *a* and *b*. The 20% underestimation of  $\Delta E(\text{PM3})$  compared to  $\Delta E(\text{SCF}, \textit{ab initio})$  stems from the underestimated monoligand Zn–water energies compared to the *ab initio* results.

The formic acid tautomer, complex *d*, is computed to have better intermolecular interaction energies than complexes *a*, *b*, and *c*. Modeling the effect of solvation with the continuum reaction field further enhances its relative stabilization. However, in terms of *total* energies now encompassing the separate intramolecular energies of formic acid and one hydroxy molecule compared to those of formate and one water molecule as computed at the MP2 level, complex *d* is the least stable *in vacuo*. Interestingly, the inclusion of solvation effects results in an equilibration of all four complexes in terms of their total energies to within < 6 kcal/mol out of 700. This implies that environmental effects, such as solvation or possibly the electrostatic potential generated by neighboring amino acid residues in the active site of a metalloenzyme, could fine-tune the coexistence of alternative tautomeric forms of the Zn ligands.

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## References

- Carrell, C. J.; Carrell, H. L.; Erlebacher, J.; Glusker, J. P. *J Am Chem Soc* 1988, 110, 8651.
- (a) Ortega-Blake, I.; Novaro, O.; Les, A.; Rybak, S. *J Chem Phys* 1982, 76, 5405; (b) Probst, M. M. *J Mol Struct (Theorchem)* 1992, 253, 275, and references therein.
- (a) Garmer, D. R.; Gresh, N.; *J Am Chem Soc* 1994, 116, 3556; (b) Gresh, N.; Stevens, W. J.; Krauss, M. *J Comput Chem* 1995, 16, 843.
- (a) Burda, J. V.; Sponer, J.; Leszczynski, J.; Hobza, P.; *J Phys Chem B* 1997, 101, 9670; (b) Sponer, J.; Burda, J. V.; Sabat, M.; Leszczynski, J.; Hobza, P. *J Phys Chem A* 1998, 102, 5951.
- Garmer, D. R.; Gresh, N.; Roques, B. P. *Proteins Struct Funct Genet* 1998, 31, 42.
- (a) Gresh, N.; Claverie, P.; Pullman, A. *Int J Quantum Chem* 1986, 29, 101; (b) Gresh, N.; Leboeuf, M.; Salahub, D. R. In *Modeling the Hydrogen Bond*, American Chemical Society Symposia 569, Smith, D. A., Ed.; American Chemical Society: Washington DC, 1994; p. 82; (c) Gresh, N.; *J Comput Chem* 1995, 16, 856; (d) Gresh, N.; Garmer, D. R. *J Comput Chem* 1996, 17, 1481; (e) Gresh, N.; *J Chim Phys* 1997, 94, 1365; (f) Gresh, N. *J Phys Chem* 1997, 101, 8680.
- Pullman, B.; Pullman, A.; Berthod, H. *Int J Quantum Chem Quantum Biol Symp* 1978, 5, 79.
- Concha, N. O.; Rasmussen, B. A.; Bush, K.; Herzberg, O. *Structure* 1996, 4, 823.
- (a) Lee, C.; Yang, W.; Parr, R. G.; *Phys Rev* 1988, B37, 785; (b) Becke, A. D. *J Chem Phys* 1993, 98, 1372.
- (a) Hartmann, M.; Clark, T.; van Eldik, R. *J Am Chem Soc* 1997, 119, 7843; (b) Pavlov, M.; Siegbahn, P. E. M.; Sandstrom, M. *J Phys Chem A* 1998, 102, 219.
- Stewart, J. J. P. *QCPE Bull* 1990, 455.
- Zheng, Y.-J.; Merz, K. M., Jr. *J Comput Chem* 1992, 13, 1151.
- Hobza, P.; Kabelac, M.; Sponer, J.; Mejlik, P.; Vondrasek, J. *J Comput Chem* 1997, 18, 1136.
- Deerfield, D. W.; Fox, D. J.; Head-Gordon, M.; Hiskey, R. G.; Pedersen, L. G. *Proteins Struct Funct Genet* 1995, 21, 244.
- Stevens, W. J.; Bash, H.; Krauss, M. *J Chem Phys* 1984, 81, 6026.
- Stevens, W. J.; Fink, W. H. *Chem Phys Lett* 1987, 139, 15.
- Schmidt, M. W.; Baldridge, K. K.; Boatz, J. A.; Elbert, S. T.; Gordon, M. S.; Jensen, J. H.; Koseki, S.; Matsunaga, N.; Nguyen, K. A.; Su, S.; Windus, T. L.; Dupuis, M.; Montgomery, J. A., Jr. *J Comput Chem* 1993, 14, 1347.
- Pople, J. A.; Binkley, J. S.; Seeger, R. *Int J Quantum Chem Symp* 1976, 10, 1.
- Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Chalamcombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. *Gaussian 94*, Revision E.2; Gaussian, Inc.: Pittsburgh, PA, 1995.
- Vigné-Maeder, F.; Claverie, P. *J Chem Phys* 1988, 88, 4934.
- Garmer, D. R.; Stevens, W. J. *J Phys Chem* 1989, 93, 8263.
- Evangelakis, G.; Rizos, J.; Lagaris, I.; Demetropoulos, G. N. *Comput Phys Commun* 1987, 46, 401.
- Langlet, J.; Claverie, P.; Caillet, J.; Pullman, A. *J Phys Chem* 1988, 92, 1631.
- (a) Langlet, J.; Gresh, N.; Giessner-Prettre, C. *Biopolymers* 1995, 36, 765; (b) Gresh, N.; Roques, B. P. *Biopolymers* 1997, 41, 145.
- Chalasinski, G.; Szczesniak, M. M. *Mol Phys* 1988, 63, 205.
- Rotzinger, F. *J Am Chem Soc* 1997, 119, 5230.
- Krauss, M.; Basch, H.; Miller, K. J. *J Am Chem Soc* 1988, 110, 4517.
- Tiraboschi, G.; Gresh, N.; Giessner-Prettre, C.; Pedersen, L. G., and Deerfield, D. W. *J Phys Chem* (submitted).
- Elrod, M. J.; Saykally, R. J. *Chem Rev* 1994, 94, 1975.
- Niesar, U.; Corongiu, G.; Huang, M.-J.; Dupuis, M.; Clementi, E. *Int J Quantum Chem Symp* 1989, 23, 421.
- Corongiu, G.; Clementi, E. *J Chem Phys* 1978, 69, 4885.
- (a) Field, M. J.; Bash, P. A.; Karplus, M. *J Am Chem Soc* 1987, 109, 8092; (b) Aqvist, J.; Warshel, A. *Chem Rev* 1993, 93, 2523; (c) Gao, J. *J Phys Chem* 1992, 96, 6432; (d) Théry, V.; Rinaldi, D.; Rivail, J.-L.; Maigret, B.; Ferenczy, G. G. *J Comput Chem* 1994, 15, 269; (e) Stanton, R. V.; Little, L. R.; Merz, K. M., Jr. *J Phys Chem* 1995, 99, 483; (f) Maseras, F.; Morokuma, K. *J Comput Chem* 1995, 16, 1170; (g) Eurenium, K. P.; Chatfield, D. C.; Brooks, B. R.; Hodoscek, M. *Int J Quantum Chem* 1996, 60, 1189; (h) Moliner, V.; Turner, A. J.; Williams, I. H. *Chem Commun* 1997, 1271; (i) Hart, J. C.; Burton, N. A.; Hillier, I. H.; Harrison, M. J.; Jewsbury, P. *Chem Commun* 1997, 1431.
- Giessner-Prettre, C.; Jacob, O. *J Comput Aided Mol Des* 1989, 3, 23.